

AD _____

Award Number: DAMD17-97-1-7236

TITLE: Identification of Tumor Suppressor Genes in Breast Cancer
by Insertional; Mutagenesis and Functional Inactivation (96
Breast)

PRINCIPAL INVESTIGATOR: Yan A. Su, M.D., Ph.D.

CONTRACTING ORGANIZATION: National Institutes of Health
Bethesda, Maryland 20892

REPORT DATE: September 1999

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are
those of the author(s) and should not be construed as an official
Department of the Army position, policy or decision unless so
designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE September 1999	3. REPORT TYPE AND DATES COVERED Annual (25 Aug 98 - 24 Aug 99)	
4. TITLE AND SUBTITLE Identification of Tumor Suppressor Genes in Breast Cancer by Insertional; Mutagenesis and Functional Inactivation (96 Breast)			5. FUNDING NUMBERS DAMD17-97-1-7236	
6. AUTHOR(S) Yan A. Su, M.D., Ph.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) National Institutes of Health Bethesda, Maryland 20892 e-mail: Suya@gunet.georgetown.edu			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited			12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 Words) The development and progression of cancer result from multiple genetic changes accumulated in the cells. The identification of tumor suppressor genes inactivated and proto-oncogenes activated in mammary epithelial cells is essential to understand the genetic basis of breast cancer and is a prerequisite for development of strategies for prevention, diagnosis, and treatment. In breast cancer, loss of heterozygosity (LOH) was detected frequently on chromosome 17 and other chromosomes, suggesting unrecognized tumor suppressor genes. We are applying the novel retroviral-tagging strategy to identify the genes using chromosome 17-suppressed (independent of p53 and BRCA1) breast cancer cell lines. In contrast to the parental tumorigenic cell line CAL51, the suppressed sublines CAL/17-1, CAL/17-3 and CAL/17-5 display insulin-dependent growth in flasks, no growth in soft-agar culture and athymic nude mice. In this annual report, we present our results on selection for the anchorage-independent cell sublines induced by retroviral transduction of the chromosome 17-mediated suppressed cell lines CAL/17, in addition to successful selection for insulin-independent cell sublines. We are now in the process to conduct tumorigenicity tests in athymic nude mice and to clone genomic sequences flanking the integrated retroviral vectors.				
14. SUBJECT TERMS Breast Cancer, IDEA Award, Tumor Suppression, Retrovirus-reversion, Anchorage- Dependent and Independent, Insulin- Dependent and Independent			15. NUMBER OF PAGES 19	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

____ Where copyrighted material is quoted, permission has been obtained to use such material.

____ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

____ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

Ys✓ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

Ys✓ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

Ys✓ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

Ys✓ In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

Ys✓ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.


PI - Signature

Sept. 21, 1999
Date

(4) TABLE OF CONTENTS

<u>Contents</u>	<u>Page Numbers</u>
(1) Front Cover	1
(2) Standard Form (SF) 298, Report Documentation Page	2
(3) Foreword	3
(4) Table of Contents	4
(5) Introduction	5
(6) Body	8
(7) Key Research Accomplishments	12
(8) Reportable Outcomes	13
(9) Conclusions	14
(10) References	15

(5) INTRODUCTION

Breast cancer is the most common malignancy in Western women, affecting up to one in 10 women during their lifetime and approximately 40,000 women dying from the disease each year in the U.S. The development of breast cancer is believed to result from multiple genetic changes accumulating in mammary epithelial cells. In searching for the genetic changes, increasing interest has been focused on tumor suppressor genes involved in protection (or control) of normal mammary epithelial cells from tumorigenic transformation.

While many investigators have observed LOH in breast cancers on chromosomes 1p, 1q, 3p, 6q, 7q, 11p, 13q, 16q, 17p, 17q, and 18q at the high frequencies variable from 20-60%, chromosome 17 is one of the most frequent carriers of LOH (1-19). On 17p, two distinct regions, 17p13.1 (containing *p53*) and 17p13.3, have shown LOH with frequencies ranging from 30-60% and 60-70%, respectively (10-11, 20-26). On 17q, three regions of frequent LOH have been identified. 17q21 LOH (13, 26-30) contains *BRCA1* (31). LOH at 17q11.1-q12 was detected as frequently as 79% in sporadic breast cancer (32). The third LOH is telomeric to *BRCA1* (26, 29). These studies demonstrate that, in addition to *p53*, *BRCA1*, *BRCA2*, and others, chromosome 17 and other chromosomes with the high frequent areas of LOH harbor unrecognized tumor suppressor genes involved in the control of the normal growth of mammary epithelial cells.

Direct evidence supporting the existence of additional breast cancer suppressor genes comes from introduction of a *neo*-tagged chromosome 17 into breast cancer cell lines by microcell-mediated chromosome transfer that demonstrated suppression of tumorigenicity (33-37). Casey *et al.* was the first to provide biological evidence that *in vitro* growth of the breast cancer cell line MCF 7 (carrying wild-type *p53*) was suppressed by the introduction of a *neo*-tagged chromosome 17 (33). A very similar result was independently reported by Negrini *et al.* (34). In addition, anchorage-independent growth, cell growth rate on plastic plates, and tumorigenicity in athymic nude mice of the mammary carcinoma cell line R30 were suppressed by introduction of chromosome 17. Wild-type *p53* was not involved in this suppression (35). Furthermore, only the long arm of the transferred chromosome 17 was capable of suppressing the tumorigenicity of the *p53*-mutant breast cancer cell line MDA-MD-231 (34). Finally, Theile *et al.* demonstrated that suppression of tumorigenicity of the breast cancer cell line CAL51 by an introduced chromosome 17 did not require transfer of *p53* or *BRCA1* (37). Thus, additional tumor suppressor genes on chromosome 17 have yet to be identified.

To facilitate identification of tumor suppressor genes we have developed a novel strategy to reverse monochromosome-mediated tumor suppression by retroviral insertional mutagenesis and/or functional inactivation mediated by expressed cDNA fragments (38). Insertional mutagenesis disrupts tumor suppressor genes (e.g., *APC* for familial adenomatous polyposis [39] and *p53* in osteosarcoma [40]) and has been used as a powerful tool to identify various genes including *Fli-1*, *p53*, *erb-B*, and *myc* (41-43), *vin-1/cyclin D2* (44), *Tiam-1* (45), *bcar-1*, and *CRL-1* (46-47). Furthermore, it is known that retroviral insertion could activate proto-oncogene. Finally, functional inactivation mediated by expressed cDNA fragments has been achieved by knockout of gene function (48) and by methods designed to identify genetic suppressor elements by antisense cDNA

or dominant negative mutant proteins (49-51). Combining all components of these established approaches into a single system of retroviral-vector-cDNA, we have developed a novel strategy for identification of genetic loci and tumor suppressor genes (38).

“Suppression and reversion of suppression” are the two basic aspects of this novel strategy. “Suppression” refers to the suppression of tumorigenic phenotypic features including anchorage-independent growth, focus formation in plastic culture, rapid cell population doubling time, and tumor formation in athymic nude mice by introduction of a *neo*-tagged monochromosome into a cancer cell line via a microcell mediated chromosome transfer. “Reversion of suppression” means the reversion to the tumorigenic phenotype induced by insertional mutagenesis (proviral tagging) and/or functional inactivation of the suppressor gene(s) by antisense or dominant negative mutant proteins following the transduction of a retrovirus expression vector-carried cDNA library into the monochromosome suppressed cells.

The hypothesis underlying this approach is the following. (1) The phenotypic reversion can derive from inactivation of tumor suppressor genes. The suppression related genetic locus or loci on the introduced chromosome provide targets to insertional mutagenesis. In addition, derived from cancer cell lines, the suppressed sublines may carry many mutated genetic loci that leave functional counterparts being “haploid” targets to insertional mutagenesis. Furthermore, the suppressor gene products can be targets to functional inactivation by antisense and mutant proteins. The poly(A)⁺-RNA from the suppressed cancer cell line is used to construct cDNA library and then re-introduced to the suppressed cell line to induce the reversion, reasoning that cDNA fragments of the suppressor genes would be present in the library providing antisense and mutant proteins to inhibit activities of the suppressor genes. It is well known that the phenotypic reversion can also come from activation of proto-oncogenes. (2) The successfully transduced tumorigenic cells can be positively selected in soft agar culture following co-selection for the drug-resistance genes on both the suppressive chromosome and the retroviral vector. (3) The cDNA and genomic sequences tagged to the vectors and involved in the tumorigenic reversion can be readily isolated by PCR-based techniques. Based on this hypothesis we have successfully generated a serial retroviral-tagged revertant cell sublines using the chromosome-6 suppressed melanoma cell line UACC903(+6) (38).

We are applying the same strategy to identify unrecognized tumor suppressor gene(s) from a chromosome-17 suppressed breast cancer cell sublines. This study is using the tumorigenic cell line CAL51 and the chromosome-17 suppressed cell sublines CAL/17-1, CAL/17-3, and CAL17-5 (37). The parental CAL51 cell line demonstrates insulin-independent growth, anchorage-independent growth, and rapid formation of subcutaneous tumors in athymic nude mice. All these readily detectable phenotypic features are suppressed in the chromosome 17 containing cell sublines CAL/17-1, CAL17-3 and CAL17-5 (37). The central goal of this project is to identify breast cancer suppressor genes. The specific aims include (1) use of the chromosome-17 suppressed breast cancer cell sublines CAL/17-1, CAL17-3 and CAL17-5 (37) to generate the anchorage-independent revertants by transduction of a retroviral expression vector-carried cDNA library and (2) use of the anchorage-independent revertants to identify previously unrecognized suppressor genes in breast cancer. We now report our results on the selection

for anchorage-independent cell sublines, in addition to successful selection for the insulin-independent cell sublines.

(6) BODY

Below are the timetable and the technique objectives of the **first-two years** from our original proposal. The current status of the technique objectives is indicated in the parentheses. The detailed description follows the Statement of Work.

Table 1. The Timetable for the Proposed Experiments

Specific Aim 1	Specific Aim 2
Year 01	
<ul style="list-style-type: none">- Completion of the library construction- Completion of the retrovirus package- Completion of the retrovirus transduction	
Year 02	
<ul style="list-style-type: none">- Completion of soft agar selection for colony forming cells and establishment of revertant sublines- Study of <i>in vitro</i> growth and tumorigenicity test- Identification of the effective cDNAs	

Technique Objectives

Task 1: Months 1-2: Cell culture of the CAL51 and CAL/17-1 cells. Isolation of poly(A)⁺RNA from the chromosome-17 suppressed cell line CAL/17-1 for construction of a cDNA library. Isolation of DNA and total RNA from the two cell sublines for the future Southern and Northern analysis. **(Completed)**.

Task 2: Months 2-3: Transfection of pLM2 plasmid vector into CAL51 and CAL/17-1 cells to determine the killing curves in the presence of L-histidinol dihydrochloride. **(Completed)**.

Task 3: Months 3-4: Soft agar culture of the L-histidinol-resistant CAL51 and CAL/17-1 sublines (transfected with pLM2 vector) to generate the first-hand data for soft agar selection experiments. **(Completed)**.

Task 4: Months 3-5: Construction of a random primed normalized cDNA library onto pLM2 vectors using poly(A)⁺RNA isolated from CAL/17-1 cells. **(Modified and completed)**.

We have constructed the randomly-primed cDNA library as reported in the previous fiscal year.

Task 5: Months 6-8: Package of the ecotropic and amphotropic retrovirus particles from the pLM2-carried cDNA library and determination of a titer of the retrovirus particles. **(Completed)**.

Task 6: Months 9-11: Transduction of the retrovirus particles into the CAL/17-1 cells and selection for colony forming cells using soft agar culture. **(Completed)**.

Task 7: Months 11-14: Individual colonies will be lifted from soft agar culture. Cells from the individual colonies will be expanded on plastic culture and re-plated in soft agar culture to eliminate false positives and to establish true revertant sublines. **(Nearly completed)**.

Colonies have been lifted from soft agar culture. The cells from the colonies are in expansion for re-plating in soft agar culture to eliminate false positives and to establish true revertant sublines.

Task 8: Months 14-16: Study of *in vitro* growth of anchorage-independent revertant cell sublines to select candidates for test of tumorigenicity in athymic nude mice. **(To be done)**.

Task 9: Months 17-20: Tumorigenicity tests of the candidate sublines in athymic nude mice to identify the tumorigenic sublines. **(To be done)**.

Task 10: Months 19-23: Identification of cDNA inserts on the integrated retroviral vectors. Test of effects of identified cDNAs on tumorigenic reversion of the CAL/17-1 cell line. **(To be done)**.

Task 11: Months 21-24: Cloning of genomic sequences flanking the integrated retrovirus vectors and subsequent identification of their encoded or adjacent cDNAs. **(To be done)**.

In addition, after the retroviral transduction, we have successfully selected for insulin-independent cell sublines from the chromosome 17-mediated suppressed cell line CAL/17-1. Since the seven insulin-independent cell sublines display the significantly different growth rates, it is likely that the retroviral vector in these cell lines are located at the different genetic loci. We are isolating their genomic DNAs for Southern blots in order to verify this view.

MATERIALS, METHODS, AND PROCEDURES

Cell Culture. The parental breast cancer cell line CAL51 and the chromosome-17 suppressed cell sublines CAL/17-1, CAL/17-3, and CAL/17-5 (37) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 100 units/mL penicillin G sodium, and 100 ug/mL streptomycin sulfate. Six hundred ug/mL of G418 and 10 ug/mL insulin were added in culture of the chromosome 17-containing cells. The insulin-independent revertant cell sublines are cultured in the absence of the insulin. Bosc23 cells (52) and Bing (53) cells were cultured in DMEM supplemented with 10% fetal bovine serum, 100 units/mL penicillin G sodium, and 100 ug/mL streptomycin sulfate. Ten% of newborn calf serum was used for culture of GP+envAM12 cells (54). Eight mM of L-histidinol dihydrochloride (hisD^R) was used for cells containing retrovirus vectors. All media, serum, and antibiotics were from Gibco BRL with exceptions where indicated. *Escherichia coli* strain DH5 α cells (GIBCO, BRL) containing plasmid pLM2 (38) and strain Supercompetent cells (Catalogue no.

230140; Stratagene) containing a cDNA library were cultured in Luria-Bertani medium with 100 ug of ampicillin per ml.

Techniques of the Molecular Cloning. Genomic DNA and total RNA were isolated by standard methods (55). Poly(A)⁺-RNA was extracted from total RNA using the FastTrack 2.0 mRNA Isolation Kit according to manufacturer's instructions (Catalog nos. K1593-02, K1593-03; Invitrogen). The cDNA library was synthesized from poly(A)⁺ RNA using Universal RiboClone cDNA Synthesis System (Catalog no. C4360; Promega) according to the manufacturer's instruction. Briefly, first strand synthesis was driven by Avian Myeloblastosis Virus (AMV) reverse transcriptase and random hexameric primers, followed directly by second strand replacement synthesis using Rnase H and DNA polymerase I. After treatment with T4 DNA polymerase to flush the ends, the double-stranded cDNA molecules were prepared for cloning by size fractionation and the addition of *Eco*RI adaptors. The resulting cDNA samples were cloned into pLM2 plasmid vectors. To generate high transformation efficiency, the double stranded cDNA were phosphorylated and pLM2 vectors were dephosphorylated before ligation. Plasmid DNA was isolated using alkaline lysis methods (56) or using QIAGEN Plasmid Purification Kit (catalogue No. 12262) according to the manufacturer's instruction.

Packaging of Retrovirus Particles. Virus particles containing retroviral vectors were packaged from pLM2 plasmids, using the retrovirus-packaging cell lines Bosc-23 (ecotropic) and GP+envAM12 (amphotropic) and BING (amphotropic) by methods essentially as described (52-54). Briefly, 50 ug of plasmid DNA or pLM2-carried cDNA library were used to transfect approximately 10⁷ Bosc-23 or BING cells using Cell-Porator Electroporation System I (catalogue No. 71600-019; GIBCO BRL) according to the manufacturer's instruction. To determine the transfection efficiency, 50-ug plasmid vectors with a green fluorescence gene was transfected into the same cells under the same conditions. 10 ml of supernatant from transfected Bosc-23 cells were also used to transduce aliquots of 1 x 10⁶ GP+envAM12 cells in the presence of 6 ug Polybrene per ml (54) for production of the amphotropic virus particles. The pLM2-virus vector-containing cells are selected for with L-histidinol. The amphotropic virus particles from packaged from the Bing cells were used to transduce the breast cancer cell lines.

Transduction. 15 T75-flasks of the chromosome 17-mediated suppressed cells (CAL/17-1) were cultured up to 50% confluence (approximately 10⁷ cells/flask) and transduced with amphotropic virus particles in the transduction medium. The aliquots of 10 ml transduction medium consist of 5 ml culture medium and 5 ml supernatant from the transduced BING cells. 4-ug polybrene were added in each ml medium to enhance the attachment of the virus particles to the cell surface. The fresh transduction medium was used to replace the old one every 4 hours per day for three days. The transduced cells were selected for with both G418 and HisD for 10 days.

Soft agar selection. Approximately 3 x 10⁶ transduced cells were cultured in 0.33% soft agarose based on 0.9% bottom agarose in the concentration of 50,000 per 60-mm dish. After 4-week culture, all the cells from the top agarose were combined for the second round of the soft agar selection to enrich the true positive anchorage-independent

cells. After the second round selection, more than 100 cell sublines are cultured, at this moment, in dishes for cell expansion and further analysis.

Selection for insulin-independent cell sublines. Approximately 5×10^6 transduced cells were cultured in the medium without insulin for two months to select for insulin-independent cell sublines. The CAL51 and CAL/17-1 cells were used as the positive and negative controls, respectively. At this moment, 7 insulin-independent cell lines are in culture for further analysis.

(7) KEY RESEARCH ACCOMPLISHMENT

This research has generated the following key research accomplishment.

- a. The pLM2 plasmid-carried cDNA library from the chromosome 17-mediated suppressed breast cancer cell line CAL/17-1 is now available for many purposes.
- b. Both the pLM2 plasmid vectors and the pLM2-carried cDNA library have been introduced into BOSC23, GP+am12, and BING cell lines for packaging the virus particles.
- c. The suppressed cell line CAL/17-1 and the control cell line CAL51 have been transduced with the packaged virus particles.
- d. More than 100 individual anchorage-independent cells have been selected from the soft agar assays. These cells are in plastic culture for cell expansion and further analysis.
- e. In addition to the accomplishment of above proposed work, we have also selected for insulin-independent cells from the transduced CAL/17-1 cells. At this moment, 7 insulin-independent cell sublines display significantly different growth rates and are in expansion for further analysis.

(8) REPORTABLE OUTCOMES

The following results are reportable in near future.

- a. The development of breast cancer cell sublines such as the anchorage-independent cell sublines and the insulin-independent cell sublines.
- b. The preparation of the abstract and manuscript on the development of these cell sublines is in progress. This will be submitted to DOD U.S. Army Medical Research and Materiel Command Breast Cancer Research Program "Era of Hope Meeting" in June 8-12, 2000.

(9) CONCLUSIONS

- a. Our preliminary results demonstrate that we have successfully applied our retrovirus-reversion strategy developed in malignant melanoma system to the chromosome 17-mediated suppressed breast cancer cell line.
- b. The resulting anchorage-independent breast cancer cell sublines will be unique and very valuable for study of breast cancer progression including the identification of genes involved in this reversion.
- c. The successful generation of the insulin-independent breast cancer cell sublines not only demonstrates another application of our retrovirus-reversion strategy but also provides unique and valuable cell resource for study of the growth factor dependent and independent biology.
- d. Our results on the successful generation of the anchorage-independent and the insulin-independent cell sublines strongly suggest that the retrovirus-reversion strategy can be applied to many areas. For example, we can select for the hormone-independent cell sublines from a hormone-independent cell line, the tumorigenic cell sublines from a non-tumorigenic cell line, and the metastatic cancer cell sublines from a non-metastatic cell line.

(10) REFERENCES

1. Genuardi M, Tsihira H, Anderson DE, Saunders GF. (1989) Distal deletion of chromosome 1p in ductal carcinoma of the breast. *Am J Hum Genet* 45: 73-82.
2. Chen L-C, Dollbaum C, Smith HS. (1989) Loss of heterozygosity on chromosome 1 in human breast cancer. *Proc Natl Acad Sci USA* 86: 7204-7.
3. Gendle, SJ, Cohen EP, Craston A, Duhig T, Johnstone G, Barnes D. (1990) The locus of the polymorphic epithelial mucin (PEM) tumour antigen on chromosome 1q21 shows a high frequency of alteration in primary human breast tumours. *Int J Cancer* 45: 431-5.
4. Devilee P, Van den Broek M, Kuipers-Dijkshoorn N, Kolluri R, Rhan PM, Cornelisse CJ. (1989) At least four different chromosomal regions are involved in loss of heterozygosity in human breast carcinoma. *Genomics* 5: 554-60.
5. Devilee P, van Vliet M, van Sloun P, Dijkshoorn NK, Hermans J, Pearson PL, Cornelisse CJ. (1991) Allelotype of human breast carcinoma: a second major site for loss of heterozygosity is on chromosome 6q. *Oncogene* 6:1705-11.
6. Bieche J, Champeme MH, Matifas F, Hacene K, Callahan R, Lidereau R. (1992) Loss of heterozygosity on chromosome 7q and aggressive primary breast cancer. *Lancet* 339: 139-43.
7. Ali IU, Lidereau R, Theillet C, Callahan R. (1987) Reduction to homozygosity of genes on chromosome 11 in human breast neoplasia. *Science (Washington DC)* 238: 185-8.
8. Lundberg C, Skoog L, Cavenee WK, Nordenskjold M. (1987) Loss of heterozygosity in human ductal breast tumors indicates a recessive mutation on chromosome 13. *Proc Natl Acad Sci USA* 84: 2372-6.
9. Radford DM, Fair KL, Phillips NJ, Ritter JH, Steinbrueck T, Holt MS, Donis-Keller H. (1995) Allelotyping of ductal carcinoma in situ of the breast: deletion of loci on 8p,13q, 16q, 17p and 17q. *Cancer Res* 55: 3399-405.
10. MacKey J, Steel CM, Elder PA, Forrest APM, Evens H-J. (1988) Allele loss on the short arm of chromosome 17 in breast cancers. *Lancet* 2: 1384-5.
11. Coles C, Thompson AM, Elder PA, Cohen BB, MacKenzie, Cranston G, Chetty U., MacKay J, Macdonald M, Nakamura Y, Hoyheim B, Steel CM. (1990) Evidence implicating at least two genes on chromosome 17p in breast carcinogenesis. *Lancet* 336: 761-3.
12. Hall JM, Lee MK, Newman B, Morrow JE, Anderson LA, Huey B, King MC. (1990) Linkage of early-onset familial breast cancer to chromosome 17q21. *Science (Washington DC)* 250: 1684-9.
13. Narod SA, Feunteun J, Lynch HT, Watson P, Conway T, Lynch J, Lenoir GM. (1991) Familial breast-ovarian cancer on chromosome 17q12-q23. *Lancet* 338: 82-3.
14. Devilee P, van Vliet M, Kuipers-Dijkshoorn N, Pearson PL, Cornelisse CJ. (1991) Somatic genetic changes on chromosome 18 in breast cancer: is the DCC gene involved? *Oncogene* 6: 311-5.

15. Dutrillaux B, Gerbault-Seureau M, Zafrani B. (1990) Characterization of chromosomal anomalies in human breast cancer. A comparison of 30 paradiploid cases with few chromosome changes. *Cancer Genet Cytogenet* 49: 203-17.
16. Mitchell EL, Santibanez-Koref MF. (1990) 1p13 is the most frequently involved band in structural rearrangements in human cancer. *Genes Chromosomes Cancer* 2: 278-89.
17. Hainsworth PJ, Raphael KL, Stillwell RG, Bennet RC, Garson OM. (1991) Cytogenetic features of twenty-six primary breast cancers. *Cancer Genet Cytogenet* 53: 205-18.
18. Sato T, Akiyama F, Sakamoto G, Kasumi F, Nakamura Y. (1991) Accumulation of genetic alterations and progression of primary breast cancer. *Cancer Res* 51: 5794-9.
19. Pandis N, Helm S, Bardi G, Idvall I, Mandahl N, Mitelman F. (1993) Chromosome analysis of 20 breast carcinomas: cytogenetic muticlonality and karyotypicpathologic correlations. *Genes Chromosomes Cancer* 6: 51-7.
20. Chen LC, Neubauer A, Kurisu W, et al. (1991) Loss of heterozygosity on the short arm of chromosome 17 is associated with high proliferative capacity and DNA aneuploidy in primary human breast cancer. *Proc Natl Acad Sci USA* 88: 3847-51.
21. Deng G, Chen LC, Schott DR, Thor A, Bhargava V, Ljung BM, Chew K, Smith HS. (1994) Loss of heterozygosity and *p53* gene mutations in breast cancer. *Cancer Res* 54: 499-505.
22. Cornelis RS, van Vliet M, Vos CB, Cleton-Jansen AM, van de Vijver MJ, Peterse JL, Khan PM, Borresen AL, Cornelisse CJ, Devilee P. (1994) Evidence for a gene on 17p13.3, distal to TP53, as a target for allele loss in breast tumors without *p53* mutations. *Cancer Res* 54: 4200-6
23. Sato T, et al. (1990) Allelotype of breast cancer: cumulative allele losses promote tumor progression in primary breast cancer. *Cancer Res* 50: 7184-9.
24. Andersen TI, Gaustad A, Ottestad L, Farrants GW, Nesland JM, Tveit KM, Barresen AL. (1992) Genetic alterations of the tumor suppressor gene regions 3p, 11p, 13q, 17p, and 17q in human breast carcinomas. *Genes Chrom Cancer* 4: 113-21.
25. Isomura M, Tanigami A, Saito H, Harada Y, Katagiri T, Inazawa J, Ledbetter DH, et al. (1994) Detailed analysis of loss of heterozygosity on chromosome band 17p13 in breast carcinoma on the basis of a high-resolution physical map with 29 markers. *Genes Chrom Cancer* 9: 173-9.
26. Kirchweber R, Zeillinger R, Schneeberger C, Speiser P, Louason G, Theillet C. (1994) Patterns of allele losses suggest the existence of five distinct regions of LOH on chromosome 17 in breast cancer. *Int J Cancer* 56: 193-9.
27. Hall JM, et al. (1992) Closing in on a breast cancer gene on chromosome 17q. *Am J Hum Genet* 50: 1235-42.
28. Cornelis RS, Devilee P, van Vliet M, Kuipers-Dijkhoorn N, Kersenmaecker A, Bardoel A, Khan PM, Cornelisse CJ. (1993) Allele loss patterns on chromosome 17q in 109 breast carcinomas indicate at least two distinct target regions. *Oncogene* 8: 781-5.

29. Cropp CS, Champeme M-H, Liderau R, Callahan R. (1993) Identification of three regions on chromosome 17q in primary human breast carcinomas which are frequently deleted. *Cancer Res* 53: 5617-9.
30. Borg A, Zhang Q-X, Johannsson O, Olsson H. (1994) High frequency of allelic imbalance at the *BRCA1* region on chromosome 17q in both familial and sporadic ductal breast carcinoma. *J Natl Cancer Inst* 86: 792-4.
31. Miki Y, Swensen J, Shattuck-Eidens D, et al. (1994) A strong candidate for the breast and ovarian cancer susceptibility gene *BRCA1*. *Science* 266: 66-71.
32. Futreal PA, Soderkvist P, Marks JR, Iglehart JD, Cochran C, Barrett JC, Wiseman RW. (1992) Detection of frequent allelic loss on proximal chromosome 17q in sporadic breast carcinoma using microsatellite length polymorphisms. *Cancer Res* 52: 2624-7.
33. Casey G, Plummer S, Hoeltge G, Scanlon D, Fasching C, Stanbridge EJ. (1993) Functional evidence for a breast cancer growth suppressor gene on chromosome 17. *Human Mol Genet* 2: 1921-7.
34. Negrini M, Sabbioni S, Haldar S, Possati, Castagrolì A, Corallini A, et al. (1994) Tumor and growth suppression of breast cancer cells by chromosome 17-associated functions. *Cancer Res* 54:1818-24.
35. Theile M, Hartmann S, Naundorf H, Rueb D, Elbe B, Krause H, Deppert W, Barrett JC, Scherneck S. (1994) Wild-type *p53* is not involved in reversion of the tumorigenic phenotype of breast cancer cells after transfer of normal chromosome 17. *Int J Oncol* 4: 1067-75.
36. Chen P, Ellmore N, Weissman BE. (1994) Functional evidence for a second tumor suppressor gene on human chromosome 17. *Mol Cell Biol* 14: 534-42.
37. Theile M, Hartmann S, Scherthan H, Arnold W, Deppert W, Frege R, Glaab F, Haensch W, Scherneck S. (1995) Suppression of tumorigenicity of breast cancer cells by transfer of human chromosome 17 does not require transferred *BRCA1* and *p53* genes. *Oncogene* 10:439-47.
38. Su YA, Ray ME, Lin T, Bodine DM, Meltzer PS, and Trent JM. (1996) Reversion of monochromosome mediated suppression of tumorigenicity in malignant melanoma by retroviral transduction. *Cancer Res* 56: in press. Reviewers, please see the attached preprint.
39. Miki Y, Mishisho I, Horii A, Miyoshi Y, Utsunomiya J, Kinzler KW, Vogelstein B, Nakamura Y. (1992) Disruption of the APC gene by a retrotransposal insertion of L1 sequence in a colon cancer. *Cancer Res* 52: 643-5.
40. Mitreiter K, Schmidt J, Luz A, Atkinson MJ, Hofler H, Erfle V, Strauss PG. (1994) Disruption of the murine *p53* gene by insertion of an endogenous retrovirus-like element (ETn) in a cell line from radiation-induced osteosarcoma. *Virology* 200: 837-41.
41. van Lohuizen, M. and Berns, A. (1990) Tumorigenesis by slow-transforming retroviruses-an update. *Bioch Biophys Acta* 1032: 213-35.
42. Peters G. (1990) Oncogenes at viral integration sites. *Cell Growth and Differentiation* 1: 503-510.

43. Ben-David Y and Bernsterns A. (1991) Friend virus-induced erythroleukemia and the multistage nature of cancer. *Cell* 66: 831-4.
44. Hanna Z, Jankowski M, Tremblay P, Jiang X, Milatovich A, Francke U, Jolicoeur P. (1993) The Vin-1 gene, identified by provirus insertional mutagenesis, is the cyclin D2. *Oncogene* 8: 1661-6.
45. Habets GGM, Scholtes EHM, Zuydgeest D, van der Kammen RA, Stam JC, Berns A, and Collard JG. (1994) Identification with an invasion-inducing gene, Tiam-1, that encodes a protein with homology to GDP-GTP exchangers for rho-like proteins. *Cell* 77: 537-49.
46. Dorssers LC, van Agthoven T, Dekker A, van Agthoven TL, Kok EM. (1993) Induction of antiestrogen resistance in human breast cancer cells by random insertional mutagenesis using defective retroviruses: identification of bcar-1, a common integration site. *Mol Endocrinol* 7: 870-8.
47. Lu SJ, Man S, Bani MR, Adachi D, Hawley RG, Kerbel RS, Ben-David Y. (1995) Retroviral insertional mutagenesis as a strategy for the identification of genes associated with cis-diamminedichloroplatinum (II) resistance. *Cancer Res* 55: 1139-45.
48. Deiss LP, Kimchi A. (1991) A genetic tool used to identify thioredoxin as a mediator of a growth inhibitory signal. *Science* 252:117-20.
49. Holzmayer TA, Pestov DG, Roninson IB. (1992) Isolation of dominant negative mutants and inhibitory antisense RNA sequences by expression selection of random DNA fragments. *Nucleic Acids Res* 20: 711-7.
50. Gudkov AV, Zelnick CR, Kazarov AR, Thimmapaya R, Suttle DP, Beck WT, Roninson IB. (1993) Isolation of genetic suppressor elements, inducing resistance to topoisomerase II-interactive cytotoxic drugs, from human topoisomerase II cDNA. *Proc Natl Acad Sci USA* 90: 3231-5.
51. Gudkov AV, Kazarov AR, Thimmapaya R, Axenovich SA, Mazo IA, Roninson IB. (1994) Cloning mammalian genes by expression selection of genetic suppressor elements: association of kinesin with drug resistance and cell immortalization. *Proc Natl Acad Sci USA* 91: 3744-8.
52. Pear WS, Nolan G P, Scott ML, Baltimore D. (1993) Production of high-titer helper-free retroviruses by transient transfection. *Proc Natl Acad Sci USA* 90: 8392-6.
53. Thony B, Leimbacher W, Stuhlmann H, Heizmann CW, Blau N. (1996) Retroviral mediated gene transfer of 6-pyruvoyl-tetrahydropterin synthase corrects tetrahydrobiopterin deficiency in fibroblasts from hyperphenylalaninemic patients. *Human Gene Therapy* 7:1587-93.
54. Markowitz D, Goff S, Bank A. (1988) Construction and use of a safe and efficient amphotropic packaging cell line. *Virology* 167:400-6.
55. Sambrook, J., Fritsch, E.F. and Maniatis, T. *Molecular Cloning* (Cold Spring Harbor Laboratory Press, Plainview, NY) pp. 7.6-7.9, 9.16-9.19, 2.108-2.117, 1989.
56. Engebrecht, J. and Brent, R. Minipreps of Plasmid DNA: Alkaline Lysis Miniprep. *In* Current Protocols in Molecular Biology, Vol. 1, Supplement 15 (F.M. Ausubel, ed.) pp.

1.6.1-1.6.2. Current Protocols: Greene Publishing Associates and John Wiley & Sons, Inc., 1991.